

## II. CLAIMS

1. (Previously Presented) In an improved method for the non-invasive early detection of colon cancer or intestinal cancer precursor cells in a sample by means of mutational analysis of two genes of each of two different signaling pathways, where the genes for the first pathway are APC, and  $\beta$ -catenin, and the genes for the second pathway are K-ras and B-raf, the improvement comprising using selected parts of the two genes in each of the signaling pathways which are combined for the analysis whereby the selection of the gene parts are determined by the following primer sequences and characterized in that where the method comprises the following steps:

collecting a stool sample,  
homogenizing the sample,  
obtaining DNA from the sample,  
performing an amplification reaction in the genes for APC,  
K-ras,  $\beta$ -catenin and B-raf,  
using the following primers for APC:  
SEQ ID NO. 1,  
SEQ ID NO. 2,  
SEQ ID NO. 3,  
SEQ ID NO. 4,  
SEQ ID NO. 5,  
SEQ ID NO. 6,  
SEQ ID NO. 7,  
SEQ ID NO. 8,  
SEQ ID NO. 9,  
SEQ ID NO. 10,  
SEQ ID NO. 15,  
SEQ ID NO. 16,

the following primers for K-ras:

SEQ ID NO. 11,

SEQ ID NO. 12,

the following primers for  $\beta$ -catenin:

SEQ ID NO. 13,

SEQ ID NO. 14,

and the following primers for B-raf:

SEQ ID NO. 17,

SEQ ID NO. 18,

wherein amplification products are formed, and performing a mutational analysis in the amplification products to determine the existence of mutations in the APC, K-ras,  $\beta$ -catenin and B-raf genes.

2. (Previously Presented) The method according to claim 1, characterized in that the detection of mutations in selected sections of the genes for APC, K-ras,  $\beta$ -catenin and B-raf is effected by means of a DNA chip, said DNA chip including probes for APC, K-ras,  $\beta$ -catenin and B-raf from those regions of the above-mentioned genes that are flanked by the primer sequences specified in claim 1.

3. (Previously Presented) The method according to claim 1, characterized in that the APC, K-ras,  $\beta$ -catenin and B-raf genes are accumulated from total DNA by hybridizing sequence-specific biotinylated oligonucleotides with the genes for APC, K-ras,  $\beta$ -catenin and B-raf using coupling of the biotin residue to streptavidin and subsequent separation via magnetic particles.

4. (Previously Presented) The method according to claim 1, characterized in that amplification products, especially PCR

products, are separated in an agarose gel for control purposes prior to purification.

5. (Previously Presented) The method according to claims 1, characterized in that the mutational analysis of the PCR products is effected using electrophoretic techniques, alternatively by means of a chromatographic procedure.

6. (Previously Presented) The method according to claim 5, characterized in that detected mutagenic conformations of a single strand are isolated and optionally sequenced.

7-8. (Canceled)

9. (Previously Presented) An improved kit, for the noninvasive early detection of colon cancer or intestinal cancer precursor cells in a sample by means of mutational analysis of two genes of each of two different signaling pathways, where the genes for the first pathway are APC, and  $\beta$ -catenin, and the genes for the second pathway are K-ras and B-raf, where the improved kit comprises selected parts of the two genes in each of the signaling pathways which are combined for the analysis where the selected gene parts are determined by the primer sequences from the group consisting of:

the following primers for APC:

SEQ ID NO. 1,  
SEQ ID NO. 2,  
SEQ ID NO. 3,  
SEQ ID NO. 4,

SEQ ID NO. 5,

SEQ ID NO. 6,

SEQ ID NO. 7,

SEQ ID NO. 8,

SEQ ID NO. 9,

SEQ ID NO. 10,

SEQ ID NO. 15,

SEQ ID NO. 16,

the following primers for K-ras:

SEQ ID NO. 11,

SEQ ID NO. 12,

the following primers for  $\beta$ -catenin:

SEQ ID NO. 13,

SEQ ID NO. 14,

and the following primers for B-raf:

SEQ ID NO. 17,

SEQ ID NO. 18,

and information relating to combining the contents of the kit and for performing a mutational analysis according to claim 1 to determine the existence of mutations in the APC, K-ras,  $\beta$ -catenin and B-raf genes.

10. (canceled)

11. (Previously Presented) A method for the detection of colon cancer or colon cancer precursor cells using the kit according to claim 9 in the method of claim 1.

12. (Previously Presented) The method of claim 5 where the

electrophoretic techniques is SSCP.

13. (Previously Presented) The method of claim 5 where the chromatographic procedure is an HPLC-based procedure.

14. (Previously Presented) The method of claim 1 using the primers SEQ ID NO. 15 and SEQ ID NO. 10 for APC, the primers SEQ ID NO. 11 and SEQ ID NO. 12 for K-ras, the primers SEQ ID NO. 13 and SEQ ID NO. 14 for  $\beta$ -catenin, and the primers SEQ ID NO. 17 and SEQ ID NO. 18 for B-raf.